Figure 2. Support for the amendment to Claim 3 can be found on page 6, lines 13-18, specifically in lines 16-18 where the number of amino acids and their corresponding location in Figure 2 is specifically disclosed. Further support for the amendment to Claim 3 and evidence that the omission of the last six amino acid residues from the sequence was a typographical error can be found in the certified English translations of the Priority Documents, particularly in Claim 2 of the translation of Japanese Patent Application No. 49636/1989. Thus, no new matter is introduced by the present Amendment.

Claims 10-13 have been added. Thus, Claims 2-7 and 10-13 are active in the present application.

REMARKS

Applicants thank Examiner Leguyader for the helpful and courteous discussion held with their U.S. representative on July 11, 1991. The following remarks will summarize and expand on topics discussed.

The present invention relates to a recombinant DNA sequence encoding physiologically active human brain natriuretic peptides (hBNP's). Neither the amino acid sequences of hBNP's nor the corresponding DNA sequences encoding them were known prior to the present invention. Accordingly, the present invention cannot be found obvious in view of the cited references, since (1) the amino acid sequence of hBNP must be known in order for the method of the

secondary reference to work, and (2) the primary reference is silent with regard to DNA, therefore it cannot provide sufficient motivation and/or enablement to one of ordinary skill in the art to prepare a recombinant DNA sequence encoding hBNP.

The rejection of Claims 2-7 under 35 U.S.C. 103 as being unpatentable over Sudoh et al in view of Suggs et al is respectfully traversed.

Sudoh et al disclose atrial natriuretic peptide (ANP), a peptide of 28 amino acids, and porcine brain natriuretic peptide (pBNP), a peptide of 26 amino acids. ANP and pBNP have 17 amino acids in common (Fig. 2, p. 80, right-hand column). Sudoh et al are completely silent with regard to DNA, as well as human BNP, and therefore, cannot possibly suggest recombinant DNA encoding human BNP.

Suggs et al disclose a method for isolating cDNA sequences, comprising chemically synthesizing a mixture of oligonucleotides that represent all possible codon combinations for a small portion of the amino acid sequence of a given protein, then probing for the desired cDNA with the mixture of oligonucleotides. Under stringent hybridization conditions, only a perfectly matched duplex will form, and since all possible codon combinations are present, one of the DNA sequences in the mixture will form a perfect match.

One key feature of the method of Suggs et al is that it allows isolation of cloned DNA sequences for a protein for which the amino acid sequence is known (p. 6613, left-hand

column, lines 9-22). Part of the strategy of the method of Suggs et al is to choose a portion of the amino acid sequence for which very few possible codon combinations exist, in order to minimize the number of oligonucleotides to be synthesized. For example, one would certainly want to select a portion of the amino acid sequence containing tryptophan (Trp) and/or methionine (Met) residues, since only one possible codon exists for each of these amino acids. Unfortunately, tryptophan and methionine are relatively rare amino acids, so one would look to a portion of the amino acid sequence containing amino acids such as asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, and tyrosine, which are encoded by two possible codons (O. Dressler and H. Potter, Discovering Enzymes, Scientific American Library Series, p. 186 (1991), submitted herewith). However, Dressler et al teach that some amino acids are coded for by as many as six possible codons. As a result, amino acid sequences having more than one amino acid with more than one or two possible codons should be avoided, as the number of possible oligonucleotide sequences encoding them can be prohibitively high.

Suggs et al disclose use of oligonucleotide probes as small as 11 bases in length, but such small oligonucleotide mixtures led to contradictory, and thus, unreliable results (p. 6616, left-hand column, line 21 through line 4 of the right-hand column). Reliable results were obtained with 15-base-long oligonucleotides.

Having the benefit of the knowledge of the amino acid sequence corresponding to the cDNA sequence for which they were probing, Suggs et al chose the amino acid sequence Trp-Asp-Arg-Asp-Met, of which 24 possible codon combinations exist. Accordingly, all 24 possible codon combinations were synthesized along with their complementary strands, and stringent hybridization of the mixture of the 24 double-stranded oligonucleotide probes (appropriately labeled) led to the isolation of the desired cDNA.

If the amino acid sequence of the protein encoded by the desired cDNA is unknown, the method of Suggs et al becomes virtually worthless. If the amino acid sequence is unknown, the possible oligonucleotide sequences are also unknown, and one must synthesize all possible codon combinations in order to hybridize the oligonucleotide to the desired cDNA. Since every possible trinucleotide combination can be a codon, one of ordinary skill in the art knows that all possible codon combinations also represents all possible oligonucleotides. As a result, the method of Suggs et al will lead to the isolation of all cDNA's having at least as many nucleotides as the oligonucleotides synthesized, which is probably going to be the same as the number of cDNA's present in the library screened. Thus, one of ordinary skill in the art would not use the method of Suggs et al to isolate cDNA encoding a polypeptide for which the amino acid sequence is unknown.

The Examiner has tried to cure this deficiency by stating that one of ordinary skill in the art would use the amino acid

sequence of pBNP to design appropriate mixtures of oligonucleotide probes encompassing all possible codon combinations for amino acid segments of pBNP. One would then use such oligonucleotide mixtures to probe for the hBNP cDNA in a human cardiac library.

In the Office Action of February 13, 1991, the Examiner stated that the polypeptide of the present Claim 8 is patentably distinct from the DNA sequences of Claims 1-7. Accordingly, the Examiner cannot make the argument that the present recombinant DNA sequences are obvious because the protein encoded by them appears to be related to a different protein disclosed by Sudoh et al. If a DNA sequence is patentably distinct from the polypeptide which it encodes, any argument against the present DNA sequences based on an assumption of similarity of a polypeptide encoded by the present DNA sequences to a polypeptide disclosed in the cited references is internally inconsistent, and must be reconsidered.

Assuming, *arguendo,* that such an argument is valid in spite of the fact that it contradicts an earlier argument put forth in support of restriction, the assertion that one of ordinary skill in the art would use the amino acid sequence of pBNP to design appropriate mixtures of oligonucleotides to probe for the hBNP cDNA requires a number of generous assumptions. First, the Examiner assumes that human BNP exists, which Sudoh et al only suggest might be possible. Sudoh et al disclose that their "identification of BNP with

Anp in porcine brain shows that a family of at least two natriuretic peptides exists in mammals" (p. 80, lines 10-15 below Table 1). Although the Examiner has interpreted the disclosure to mean that all mammals, and specifically humans, have BNP, the disclosure can also mean that some but not all mammals have BNP, or that another natriuretic peptide exists in mammals which is not necessarily BNP.

Second, the Examiner assumes that pBNP and hBNP share a completely homologous pentapeptide sequence. This is by no means a certainty, especially since the amino acid sequence of hBNP was unknown prior to the present invention.

Third, the Examiner assumes that human atrial natriuretic peptide (hANP) will not accidentally give a false positive result, considering the gene encoding hANP is highly homologous with pBNP, and which by the Examiner's reasoning, must then also be highly homologous with the gene encoding hBNP. If a pentapeptide sequence of hBNP is homologous with pBNP only at the same residues where pBNP is homologous with hANP, the method of Suggs et al will suffer problems with interference by the gene encoding hANP.

Fourth, the Examiner assumes that hBNP cDNA will be found in a human cardiac library. Although Sudoh et al disclose that "it is probable BNP is also present in other organs, such as heart," (p. 80, left-hand column, last three lines, and right-hand column, first line) this is no guarantee that the cDNA encoding hBNP is in a human cardiac library. For example, human pituitary hormones are found in many parts of

the body other than the pituitary, but the cDNA encoding human pituitary hormones is likely to be found only in a human pituitary or human brain library.

The Examiner appears to be making the argument that one could have chosen <u>any</u> peptide sequence of pBNP not homologous with hANP, constructed a reasonable number of oligonucleotide probes encoding the peptide sequence, then screened a human cardiac library for the desired cDNA with a reasonable likelihood of success. Even granting the generous assumptions above, this is still simply not true.

Assuming that hBNP exists and that there is a homologous pentapeptide sequence which does not coincide with that of hANP, how is one to know which pentapeptide sequence of pBNP is homologous with hBNP, since the amino acid sequence of hBNP was unknown prior to the present invention? To use the method of <u>Suggs et al</u>, one must try <u>all</u> pentapeptide sequences which are not homologous with hANP. How many possible codons must then be made, and would one of ordinary skill in the art consider the number within the realm of routine experimentation?

The amino acid sequence of pBNP is as follows:

Asp-Ser-Gly-Cys-Phe-Gly-Arg-Arg-Leu-Asp-Arg-Ile-Gly-
Ser-Leu-Ser-Gly-Leu-Gly-Cys-Asn-Val-Leu-Arg-Arg-Tyr

Since pBNP and hANP are homologous at positions 16-21, the oligonucleotides encoding the pentamers 16-20 and 17-21

will certainly lead to isolation of cDNA encoding hANP, so these two pentapeptide sequences can be ignored. Otherwise, all possible pentapeptide sequences of pBNP must be examined, since, at the time of the invention, the amino acid sequence of hBNP was unknown.

For example, the pentapeptide pBNP[1-5], Asp-Ser-Gly-Cys-Phe, has 192 possible oligonucleotide codon combinations. This was calculated as follows:

Asp = 2 possible codons

Ser = 6 possible codons

Gly = 4 possible codons

Cys = 2 possible codons

Phe = 2 possible codons

Hence, the possible number of oligonucleotide sequences encoding pBNP[1-5] is the number of possible codons for each amino acid times each other. In this case, the number is (2 x 6 x 4 x 2 x 2), or 192. Already, this is 8 times the largest number of possible oligonucleotides suggested by Suggs et al, and this is merely for the first of 20 possible pentamers which could give rise to hBNP without obtaining hANP. As it turns out, pBNP[1-5] has a relatively small number of possible oligonucleotide codon combinations. The calculation of the number of possible oligonucleotides encoding a suitable pentapeptide sequence of pBNP is as follows:

Pentapeptide	Codon Combinations	
1-5	(2 x 6 x 4 x 2 x 2)	= 192
2-6	(6 x 4 x 2 x 2 x 4)	= 288
3-7	(4 x 2 x 2 x 4 x 6)	= 288
4-8	(2 x 2 x 4 x 6 x 6)	= 576
5-9	(2 x 4 x 6 x 6 x 6)	= 1728
6-10	(4 x 6 x 6 x 6 x 2)	= 1728
7-11	(6 x 6 x 6 x 2 x 6)	= 2592
8-12	(6 x 6 x 2 x 6 x 3)	= 1296
9-13	(6 x 2 x 6 x 3 x 4)	= 864
10-14	(2 x 6 x 3 x 4 x 6)	= 864
11-15	(6 x 3 x 4 x 6 x 6)	= 2592
12-16	(3 x 4 x 6 x 6 x 6)	= 2592
13-17	(4 x 6 x 6 x 6 x 4)	= 3456
14-18	(6 x 6 x 6 x 4 x 6)	= 5184
15-19	(6 x 6 x 4 x 6 x 4)	= 3456
18-22	(6 x 4 x 2 x 2 x 4)	= 288
19-23	(4 x 2 x 2 x 4 x 6)	= 288
20-24	(2 x 2 x 4 x 6 x 6)	= 576
21-25	(2 x 4 x 6 x 6 x 6)	= 1728
22-26	(4 x 6 x 6 x 6 x 2)	= 1728
	Total	= 32,304

Thus, one would have to synthesize more than 64,000 oligonucleotide sequences in order to probe for cDNA encoding hBNP according to the method of Suggs et al. Thus, one would need to employ more than three orders of magnitude more oligonucleotide sequences than the largest number suggested by Suggs et al. This is clearly not a reasonable number, and is clearly outside the realm of routine experimentation.

The Examiner points to amino acid residues 20-26 as an appropriate non-homologous region for construction of a probe by the method of Suggs et al. The Examiner has not provided a reason why heptapeptide pBNP[20-26] was chosen over any other heptapeptide not homologous with hANP. Without the knowlege of the hBNP amino acid sequence, one has no reason whatsoever to choose one given sequence of pBNP over another. Further,

pBNP[20-26] alone would require the synthesis of nearly 14,000 oligonucleotide sequences. Using this sequence as a rough average, in combination with the 19 other suitable heptapeptide sequences of pBNP, one would have to synthesize approximately 560,000 oligonucleotides. This is clearly unreasonable.

It is especially interesting to note that the heptapeptide sequence chosen by the Examiner would not work. Although amino acids 17-22 of formula (I) on p. 3 of the present specification are homologous with amino acid residues 20-25 of pBNP, amino acid 23 of formula (I), His, is not homologous with amino acid residue 26 of pBNP, Tyr. Since Suggs et al teach that only perfect base pairing will lead to success, the sequence suggested by the Examiner is doomed to failure.

It cannot be emphasized strongly enough that a reference which discloses the amino acid sequence of a peptide cannot possibly suggest a recombinant DNA sequence which encodes a different polypeptide having an unknown sequence, no matter how closely related the Examiner assumes the peptides to be. After all, if something is unknown in the art at the time an invention is made, an assertion that one of ordinary skill in the art would try a method which requires knowlege of the unknown information must be erroneous. Since the methods of the cited references require some information about the amino acid sequence of the desired protein in order to isolate DNA encoding it, the argument by the Examiner that one of ordinary

skill in the art would be motivated to use the methods of the cited references to arrive at the present invention cannot form a sustainable ground of rejection.

Furthermore, since the present Inventors did not use the methods suggested by the Examiner to arrive at the present invention, the Examiner appears to be applying the "obvious to try" standard of patentability. In other words, the Examiner seems to feel that the methods disclosed in the cited references would have a reasonable likelihood of success, and thus, been "obvious to try", even though the present Inventors did not follow these methods, and even though there is considerable doubt regarding the likelihood of success using the methods.

For example, the Examiner states on page 3 of the Office Action that the method of Sudoh et al can be used to isolate substantially pure hBNP from human brain tissue, and corresponding probes can be constructed from this amino acid sequence to isolate hBNP cDNA from a human brain library. This argument contradicts the earlier statement that a DNA sequence is an independant and distinct invention from the polypeptide which it encodes, made in support of restriction. Assuming, *arguendo,* that the statements by the Examiner are not contradictory, a few critical details regarding the method of Sudoh et al have been overlooked, some of which require information about the target protein.

For instance, Sudoh et al isolate a whopping 1.5 μg of pBNP from 200 porcine brains, or about 15 nanograms of BNP per

brain. The amount of human BNP in a human brain is unknown, but even assuming it is similar to the amount of pBNP in a pig brain, there will undoubtedly be some difficulties in acquiring 200 human brains for scientific research.

Further, the first steps in the isolation of pBNP by Sudoh et al are acidic extraction, then gel filtration using 1 M AcOH, followed by pyridine and 2 M AcOH (p. 79, caption to Fig. 1, lines 2-8 and **Methods**, lines 3-7). If the gel filtration is to be successful, the size of the desired protein to be isolated should be about the same as the known protein successfully isolated. Further, use of acidic eluants requires that the desired protein have about the same basicity as the known protein. However, if the amino acid sequence of the desired protein is unknown, how can one know with any certainty whether the size and the basicity of the desired protein is the same as or similar to the known protein successfully isolated?

Similarly, the extensive chromatographic procedures reported by Sudoh et al require repeated elution using either an ammonium formate gradient or a slihgtly acidic acetonitrile gradient (p. 79, caption to Fig. 1, lines 16-26 and 30-37, and **Methods**, lines 7-10). In order for the chromatographic procedures to be successful, the desired protein must not only have the same size and basicity as the known protein (as discussed above for extraction and gel filtration), but must also have the same isoelectronic point as the known protein,

since chromatography with a salt gradient is used. Without knowlege of the amino acid sequence of the desired protein, substantial uncertainty exists as to the likelihood of success for this method in the isolation of hBNP.

In other words, for the method of Sudoh et al to have a reasonable likelihood of success in the isolation of hBNP, the size, basicity and polarity of the hBNP polypeptide must have been known prior to the present invention. The mere suggestion that a second natriuretic peptide exists in mammals does not provide enough information to support a reasonable likelihood of success for the Examiner's assertions that one of ordinary skill in the art would be motivated to isolate hBNP by the same method. Furthermore, the statement by Sudoh et al that "shorter or longer BNP-related peptides are probably present in other organs" (p. 80, right-hand column, lines 9-11 below Fig. 2) considerably clouds any certainty regarding the certainty of isolating an unknown polypeptide from a difficult-to-obtain source.

Furthermore, two very important aspects of the method used by the present Inventors to arrive at the present invention have been overlooked by the Examiner. The present specification on page 4, lines 11-20 discloses isolation of mRNA from human tissues which are thought to contain human BNP. By contrast, Sudoh et al disclose isolation of pBNP *per se* from pig brains. It is irrelevant to assert what one of ordinary skill might have done to arrive at the present

invention when the present Inventors did not use the asserted method. Since one of ordinary skill in the art would not expect a procedure used to isolate a polypeptide to be useful in the isolation of corresponding mRNA as well, the assertions by the Examiner are groundless.

Since Sudoh et al are silent with regard to DNA, they cannot possibly suggest a method for isolating a DNA sequence. Accordingly, the disclosure of Sudoh et al cannot make the present recombinant DNA sequence obvious. The Examiner appears to be reading at least part of the present disclosure (the amino acid sequence of hBNP) into the prior art (which is silent with regard to hBNP), thus using the present specification against the present invention. Accordingly, this ground of rejection is unsustainable, and should be withdrawn.

Therefore, this application is in condition for allowance. Early notice to that effect is earnestly solicited.

Respectfully submitted,

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